BIOMATERIALS BASED ON HYALURONIC ACID FOR THE ANTI-ANGIOGENIC THERAPY IN THE TREATMENT OF TUMOURS

SUBJECT OF THE INVENTION

The present invention relates to the use in the medical-surgical field of biomaterials based on hyaluronic acid derivatives, optionally in association with natural, synthetic or semisynthetic biopolymers, for suppressing the angiogenic process associated with tumour proliferation (in primary and secondary tumours).

BACKGROUND OF THE INVENTION

The induction and development of angiogenesis is a pre-requisite for the development of a primary tumour, and for any subsequent metastases.

Angiogenesis is a dynamic process closely linked with the proliferation of cancer cells, because it is the latter that are chiefly responsible for the production and release of angiogenic factors, such as cytokines and other trophic factors. An increase in the vascularisation of a primary tumour can cause an increase in the number of cancer cells that enter into the circulation and give rise to new metastases.

Recent studies have demonstrated that an increase in the density of microvessels in an area affected by neoplasia indicates new tumour growth.

It is therefore clinically important to suppress angiogenesis to inhibit its development, if possible. Indeed, by associating anti-angiogenic therapy with "classic" anticancer therapy with drugs and/or radiation, with or without surgical removal of the tumour, it is possible to halt the proliferation of cancer cells, thus preventing the invasion of further tissues by said cells, and the consequent development of new metastases (Skobe H. et al., Nature Medicine, 1222-1227 (1997)).

In histological assessment of the onset of the angiogenic process

associated with a cancerous growth, it is important to look for markers of the tumour's vascular system, for example with antibodies that differentiate the endothelial cells from the cancerous ones. For example, the anti-CD3 antibody is specific for marking the endothelial cells and therefore enables their identification in the angiogenic process associated with the development of new metastases. Its use has proved essential in assessing the level of microvessel development associated with neoplasia. Indeed, thanks to antibody marking, it is possible to visualise and count the number of interconnections of the vessels within the cancerous tissue to understand and quantify the angiogenic process, relating it to any new developments in the neoplasia (thereby deciding if/how much/how to associate a therapy that modulates or inhibits angiogenesis with an established/classic anticancer therapy.

One such therapy consists in administering drugs that act by blocking the receptors of the trophic factors (PGDF, bFGF, VEGF) that are also angiogenic factors.

Preclinical results 'in vivo' have shown that said drugs prove important in inhibiting tumour growth but they do not determine regression of the tumour itself: on the strength of these major experimental data, the drugs have been introduced in numerous clinical trials.

However, an anti-angiogenic clinical therapy that provides for a generally oral pharmacological administration in chronic form may have many toxic side effects, because angiogenesis is not only associated with pathological disorders but also physiological processes such as tissue reproduction and repair ("*Cancer: Principle Practice of Oncology*" V. De Vita, S. Hellmann and S. Rosenberg, 6th Edition).

It is therefore of strategic importance to associate classic anticancer therapy with an anti-angiogenic therapy "in situ", and this is the subject of the

present invention.

Hyaluronic acid is one of the chief components of the extracellular matrix of the connective tissue, and there are numerous scientific publications concerning its role in various processes, both physiological and pathological, such as the formation of granulation tissue, chemotaxis in the inflammatory process, cell differentiation for various cell types. Other studies concern its role within the family of "substrate adhesion molecules".

Hyaluronic acid has been used for the above indications:

- as a differentiating agent in therapy for acute myeloid leukaemia (Charrad R. S. et al., Nature Medicine 5, 669-676 (1999));
- as a vehicle for drugs such as steroids or NSAIDs, antibiotics and anti-neoplastic agents, because of the abundant expression of its receptor (CD44) in cancer cells; (Freemantle, C. et al., Int. J. Tiss. Reac. XVIII (4) 157-166 (1995); Coradini, D. et al., Int. J. Cancer 5, 411-416 (1999));
- in preclinical studies on the inhibition of lung metastasis, because of its capacity for inhibiting the adhesion of cancer cells to the vascular endothelium (Karasaza K. et al., Clinical & Experimental Metastasis 15, 83-93 (1997));
- as a means of controlling adhesion to the substrate with subsequent proliferation of cells (possibly also cancer cells) permanently "in situ" after surgical removal of tissues (including tumours) (U.S. 5,627,162).

Experimental observations "in vivo" have, however, revealed that hyaluronic acid may have a chemotaxic activity on cancer cells within the granulation tissue that forms after removal of cutaneous metastasis of melanoma (Salmon-Ehr, V. et al., Ann. Dermatol. Venereol, 123, 194-195 (1996)). Moreover, numerous pre-clinical studies have demonstrated that

hyaluronic acid enhances cancer cell migration, thereby favouring metastasis, as it is known that the degradation products of hyaluronic acid, that is, oligosaccharides constituted by 10 and 20 oligomers, are strong inducers of the angiogenic process (Hayen et al., J. Cell. Sci. 112, 2241-2251 (1999); Slevin, M. et al., Lab. Invest. 78(8), 987-1003 (1998)).

Moreover, biomaterials based on hyaluronic acid and/or the derivatives thereof have never been used as an anti-angiogenic therapy, neither have any other biodegradable and/or non-biodegradable biopolymers ever been used in anticancer therapies.

Absolutely innovative, therefore, is the use of biomaterials based on hyaluronic acid derivatives such as Hyaff® (EP 0 216 453 B1) or ACPs (EP 0 341 745 B1) in the form of non-woven felts for instance (EP0 618 817 B1) or as three-dimensional structures (WO 99/61080), possibly in association with various biomaterials (e.g. natural ones such as collagen, cellulose, polysaccharides, chitin, chitosan, pectin, agar, gellan and alginic acid, synthetic ones such as polylactic acid (PLA), polyglycolic acid (PGA), polyurethanes and polysulphonic resins, or semisynthetic ones such as collagen cross-linked with aldehyde, diamine and gellan) as a therapy to suppress and/or inhibit the angiogenic process that enhances and determines tumour metastasis.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to biomaterials based on hyaluronic acid derivatives made into non-woven felts (as the preferred form of biomaterial), optionally in association with natural, synthetic or semisynthetic biopolymers, for use in the medical-surgical field as a new anti-angiogenic therapy ("in situ"), optionally associated with classic pharmacological anticancer therapies and/or radiotherapy, to modulate indirectly the proliferation of tumours, thus blocking the formation of local relapses and, therefore, any new metastases.

In order to study, characterise and then assess "in vivo" the effect of the biomaterial of the present invention in the angiogenic process that supports the development of skin carcinomas (considered to be a clarifying example), the Applicant has developed a new model of tumour/ stromal cell support interaction, described as follows:

1) two cell lines of human keratinocytes transfected with the ras-oncogene: HACaT II-4, malignant variant and A5, benign variant;
2) said cells are transferred onto a collagen gel mounted into teflon rings covered by a silicone chamber, known as the Fusenig silicone chamber (FSC);
3) said FSC is then placed over the muscle fascia of the backs of nude mice, in the presence or absence of an immediately underlying layer of biomaterial based on Hyaff® 11 (total benzyl ester of hyaluronic acid) made in the form of a non-woven felt;
4) four to six weeks later, two different types of granulation tissue will have formed underneath the cancerous epithelium;
5) the development of the epithelial tumour and of the underlying granulation tissue is assessed, over time, both by classic histological analyses (haematoxylin/eosin) and by immunohistochemical techniques using the anti-CD31 antibody, to visualise the presence of vascular epithelium and therefore determine the development of the angiogenic process;
6) the levels of cellular proliferation are examined using immunohistochemical techniques associated with the introduction of BrdU into the DNA of proliferating cells, both within the granulation tissue underneath the epithelium and in the cancerous epithelium itself. Marking with the anti-integrin α6 antibody was also assessed to study the level of cellular proliferation within the

cancerous epithelium.

The results of the experiment were as follows:

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After 4-6 weeks, the cancerous epithelium in the control FSC (i.e. without any biomaterial placed under the epithelium), was well developed and multilayered, while the layer of granulation tissue underneath had completely replaced the layer of collagen that separated the epithelium from the underlying tissue (Fig. 1).

Conversely, four weeks later, the cancerous epithelium in the FSC placed over the Hyaff®-based biomaterial in the form of a non-woven felt is less developed than the relative control, and the layer of collagen that separates it from the nascent granulation tissue underneath is still thick and not infiltrated by cells and/or vessels (Fig. 1).

After six weeks, the quantity of collagen is still abundant, with just an initial layer of granulation tissue that begins to form over the biomaterial (Fig. 1).

HACaT II 4 line:

After four to six weeks, in the control FSC, the cancer cells have constituted a thick epithelium that penetrates into the thickness of the new granulation tissue underneath, that has already completely replaced the layer of collagen that separated it from the epithelium (Fig. 2).

Four weeks later, in the FSC placed over the Hyaff®-based biomaterial, the cancerous epithelium is thin but easily distinguishable from the granulation tissue forming over the biomaterial, separated from this tissue by the collagen gel that is still present and not yet absorbed (Fig. 2).

Six weeks later, the tumour mass and the granulation tissue have established close contact, but there has been no actual infiltration of tumour cells into the granulation tissue, unlike the control, where the tumour cells

have completely invaded the new, underlying granulation tissue (Fig. 2).

Using immunohistochemical techniques linked with the specific marking of particular nucleotides such as BrdU, at the 1st and 2nd weeks, good cell proliferation is evident within the nascent granulation tissue in the control and in the Hyaff®-based biomaterial, while at 4, and especially at 6, weeks after transplant, the cell growth rate drops drastically in the granulation tissue underneath the cancerous epithelium, which conversely maintains in both samples a good level of cellular proliferation (Fig. 3).

The growth of cancerous epithelium can also be visualised with a specific antibody against the protein integrin α6. Said molecule is, indeed, a component of the hemidesmosomes and its expression is normally only associated with the proliferative area of the epithelial layers.

Fig. 3a shows that antibody marking for the integrin protein α6 is notably present throughout the cancerous epithelium both in the control FCS and in the FCS with the Hyaff®-based biomaterial, even though expression of the test protein appears less extensive throughout the thickness of the cancerous epithelium in the latter sample.

Specific marking for the vascular epithelium with the anti-CD31 antibody reveals, furthermore, that in the controls, after four weeks, the angiogenic process is well established as the vessels in the underlying granulation tissue already reach the cancerous epithelium and after 6 weeks they invade it, thus favouring metastasis (Fig. 4).

In the case of the FSC with the Hyaff®-based biomaterial, after four weeks there is still no close contact between granulation tissue and cancerous epithelium. This will occur only after six weeks, even though there is no invasion of the epithelium by the underlying microvessels, that remain relegated to the granulation tissue (Fig. 4).

The angiogenic process seems to be at a standstill, no longer enhancing

tumour development. Vascularisation is limited to the area covered by the Hyaff®-based biomaterial, so the tumour cells do not invade the granulation tissue that has formed within the biomaterial.

In conclusion, the Hyaff®-based biomaterial proved able to modulate/inhibit the angiogenic process related to vascularisation of the cancerous epithelium. It therefore proves to be particularly advantageous to use the biomaterials based on hyaluronic acid derivatives in the oncological field, where it is important to modulate the angiogenic process and therefore, indirectly, the proliferation of cancer cells in primary and secondary tumours.

According to the invention, the biomaterials that can be useful in the oncological field as a new anti-angiogenic therapy "in situ" may be, for example, in the form of non-woven felts, sponges, films, membranes, microspheres or in other three-dimensional forms in cases where it is necessary to fill the cavities that are liable to form after surgical removal of a tumour.

The anti-angiogenic action of the biomaterial can, moreover, be supported by supplementing the biomaterial with NSAIDs, steroids, hormones, antibiotics and especially anti-cancer drugs such as fluorouracil, methotrexate, cis-platinum, carboplatin, oxaliplatin, ethopoxide, cyclophosphamide, vincristine, doxorubicin.

The invention being thus described, it is clear that these methods can be modified in various ways. Said modifications are not to be considered as divergences from the spirit and purposes of the invention and any modification that would appear evident to an expert in the field comes within the scope of the following claims.

CLAIMS

1. Biomaterials constituted by at least one hyaluronic acid derivative, optionally in association with other natural, synthetic and/or semisynthetic biopolymers and with pharmacologically active substances, as an anti-angiogenic therapy to treat primary and secondary tumours.

2. Biomaterials according to claim 1, wherein the hyaluronic acid derivative is a benzyl ester.

3. Biomaterials according to claim 1, wherein the hyaluronic acid derivative is cross-linked.

4. Biomaterials according to claim 1, wherein the natural biopolymer is selected from the group consisting of collagen, cellulose, polysaccharides, chitin, chitosan, pectins, agar, gellan and alginic acid.

5. Biomaterials according to claim 1, wherein the synthetic biopolymer is selected from the group consisting of polylactic acid (PLA), polyglycolic acid (PGA), polyurethanes and polysulphonic resins.

6. Biomaterials according to claim 1, wherein the semisynthetic biopolymer is selected from the group consisting of collagen cross-linked with aldehydes, diamine and gellan.

7. Biomaterials according to claim 1, wherein the biopolymer may optionally be in association with pharmacologically active substances such as fluorouracil, methotrexate, cis-platinum, carboplatin, oxaliplatin, ethopoxide, cyclophosphamide, vincristine, doxorubicin.

8. The use of biomaterials constituted by at least one hyaluronic acid derivative, optionally in association with other natural, synthetic and/or semisynthetic biopolymers and with pharmacologically active substances, as an anti-angiogenic therapy for the treatment of primary and secondary tumours.

9. The use of biomaterials according to claim 8, wherein the hyaluronic acid derivative is a benzyl ester.

10. The use of biomaterials according to claim 8 wherein the hyaluronic acid derivative is cross-linked.

11. The use of biomaterials according to claim 8 wherein the natural biopolymer is selected from the group consisting of collagen, cellulose, polysaccharides, chitin, chitosan, pectins, agar, gellan and alginic acid.

12. The use of biomaterials according to claim 8 wherein the synthetic biopolymer is selected from the group consisting of polylactic acid (PLA), polyglycolic acid (PGA), polyurethanes and polysulphonic resins.

13. The use of biomaterials according to claim 8 wherein the semisynthetic biopolymer is collagen cross-linked with aldehydes, diamine and gellan.

14. The use of biomaterials according to claim 8 wherein the biopolymer may optionally be in association with pharmacologically active substances such as fluorouracil, methotrexate, cis-platinum, carboplatin, oxaliplatin, ethopoxide, cyclophosphamide, vincristine and doxorubicin.

15. The use of a biomaterial according to claim 8 wherein the hyaluronic acid derivative is made into a non-woven felt, sponge, microsphere, film, membrane and/or other three-dimensional structures.

16. The use of biomaterials constituted by at least one hyaluronic acid derivative, optionally in association with other natural, synthetic and/or semisynthetic biopolymers and with pharmacologically active substances, for the treatment and care of primary and secondary tumours when the tumour has been surgically removed and the cavity that is thus formed requires filling, making it advantageous to prevent and/or inhibit the angiogenic process using the biomaterial itself.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/00078

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/00 A61P35/00 A61K47/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, MEDLINE, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 02 18448 A (FRANCESCANGELI ANDREA ;BELLINI DAVIDE (IT); CRESCENZI VITTORIO (IT) 7 March 2002 (2002-03-07) page 3, line 9-12 claims 5,11,15,24 ---	1-16
P,X	WO 02 41877 A (CLEAR SOLUTIONS BIOTECH INC ;DEHAZYA PHILIP (US); LU CHENG (US)) 30 May 2002 (2002-05-30) page 4, line 19-22 page 18, line 8 page 16, line 21 ---	1-16
P,X	WO 02 18450 A (FRANCESCANGELI ANDREA ;RENIER DAVIDE (IT); CRESCENZI VITTORIO (IT)) 7 March 2002 (2002-03-07) page 2, line 10-12 page 10, line 27 ---	1-16
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 341 745 A (FIDIA SPA) 15 November 1989 (1989-11-15) page 1, line 32 page 5, line 41-49 page 6, line 19-28 page 9, line 55 page 10, line 11 page 10, line 14	1-7
X	WO 99 61080 A (CALLEGARO LANFRANCO ;DONA MASSIMO (IT); FIDIA ADVANCED BIOPOLYMERS) 2 December 1999 (1999-12-02) page 1, line 6-13 page 4, line 23-28	1-7
X	US 5 520 916 A (CALLEGARO LANFRANCO ET AL) 28 May 1996 (1996-05-28) column 1, line 8-13 claim ALL	1-7
X	WO 00 01733 A (BELLINI DAVIDE ;FIDIA ADVANCED BIOPOLYMERS SRL (IT); TOPAI ALESSAN) 13 January 2000 (2000-01-13) page 1, line 11-14 claims 1,5,16,21,23	1-16
X	WO 00 54762 A (UNIV BOSTON ;MOULTON STEVEN (US)) 21 September 2000 (2000-09-21) claims 1-31	1-16
X	WO 00 57896 A (MUMMERT MARK E ;TAKASHIMA AKIRA (US); UNIV TEXAS (US); MOHAMADZADE) 5 October 2000 (2000-10-05) page 3, line 21-32	1-16
X	US 4 851 521 A (DELLA VALLE FRANCESCO ET AL) 25 July 1989 (1989-07-25) column 1, line 6-12 claims 1-8,25	1-16
X	EP 0 466 300 A (BIOMATRIX INC) 15 January 1992 (1992-01-15) page 3, line 35-47 page 7, line 28-31	1-16
P,X	TONELLO C ET AL: "In vitro reconstruction of human dermal equivalent enriched with endothelial cells" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 24, no. 7, March 2003 (2003-03), pages 1205-1211, XP004401497 ISSN: 0142-9612 abstract	1-7

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	GLASS J ET AL: "A three-dimensional cell attachment matrix created by cross-linking RGD peptide modified hyaluronic acid" JOURNAL OF CELLULAR BIOCHEMISTRY - SUPPLEMENT, WILEY-LISS, US, no. SUPPL 19A, 5 January 1995 (1995-01-05), page 178 XP002112805 ISSN: 0730-2312 abstract	1-7
X	LUO Y ET AL: "Cross-linked hyaluronic acid hydrogel films: new biomaterials for drug delivery" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 69, no. 1, 3 October 2000 (2000-10-03), pages 169-184, XP004217543 ISSN: 0168-3659 abstract	1-7
X	CORADINI D ET AL: "Hyaluronic acid as drug delivery for sodium butyrate: improvement of the anti-proliferative activity on a breast-cancer cell line." INTERNATIONAL JOURNAL OF CANCER. JOURNAL INTERNATIONAL DU CANCER. UNITED STATES 5 MAY 1999, vol. 81, no. 3, 5 May 1999 (1999-05-05), pages 411-416, XP002245583 ISSN: 0020-7136 abstract	1-16

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/00078

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0218448	A	07-03-2002	IT	PD20000208 A1	28-02-2002
			AU	9181501 A	13-03-2002
			WO	0218448 A2	07-03-2002
WO 0241877	A	30-05-2002	AU	3969702 A	03-06-2002
			WO	0241877 A1	30-05-2002
			US	2003096734 A1	22-05-2003
WO 0218450	A	07-03-2002	IT	PD20000207 A1	28-02-2002
			AU	1387002 A	13-03-2002
			WO	0218450 A1	07-03-2002
			EP	1313772 A1	28-05-2003
EP 0341745	A	15-11-1989	IT	1219587 B	18-05-1990
			AT	115590 T	15-12-1994
			AT	195534 T	15-09-2000
			AU	631125 B2	19-11-1992
			AU	3574789 A	29-11-1989
			CA	1339122 C	29-07-1997
			DE	68919900 D1	26-01-1995
			DE	68919900 T2	11-05-1995
			DE	68929241 D1	21-09-2000
			DE	68929241 T2	05-04-2001
			DK	10990 A	12-03-1990
			WO	8910941 A1	16-11-1989
			EP	0341745 A1	15-11-1989
			EP	0614914 A2	14-09-1994
			ES	2064378 T3	01-02-1995
			ES	2151910 T3	16-01-2001
			FI	107050 B1	31-05-2001
			GR	3015035 T3	31-05-1995
			GR	3034651 T3	31-01-2001
			HU	53666 A2	28-11-1990
			IL	90274 A	12-09-1996
			JP	10324701 A	08-12-1998
			JP	2504163 T	29-11-1990
			JP	2941324 B2	25-08-1999
			NZ	229100 A	28-08-1995
			US	5676964 A	14-10-1997
WO 9961080	A	02-12-1999	IT	PD980131 A1	29-11-1999
			IT	1302535 B1	05-09-2000
			AT	227589 T	15-11-2002
			AU	748303 B2	30-05-2002
			AU	4368099 A	13-12-1999
			CA	2332532 A1	02-12-1999
			DE	69903945 D1	19-12-2002
			DK	1085917 T3	10-03-2003
			WO	9961080 A1	02-12-1999
			EP	1085917 A1	28-03-2001
			ES	2184460 T3	01-04-2003
			JP	2002516154 T	04-06-2002
US 5520916	A	28-05-1996	IT	1254704 B	09-10-1995
			AT	200630 T	15-05-2001
			AU	669147 B2	30-05-1996
			AU	3346693 A	19-07-1993
			BG	98863 A	31-05-1995

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5520916 A		BG 302 Y1	31-05-1999
		CA 2126085 A1	24-06-1993
		DE 69231796 D1	23-05-2001
		DE 69231796 T2	15-11-2001
		DK 618817 T3	30-07-2001
		WO 9311803 A1	24-06-1993
		EP 0618817 A1	12-10-1994
		ES 2155832 T3	01-06-2001
		FI 942894 A	18-08-1994
		GR 3036197 T3	31-10-2001
		HU 68680 A2	28-07-1995
		JP 7502430 T	16-03-1995
		NO 942330 A	17-08-1994
		NZ 246575 A	24-04-1997
		PT 618817 T	30-08-2001
		RO 115017 B1	29-10-1999
		RU 2133127 C1	20-07-1999
		US 5824335 A	20-10-1998
WO 0001733 A	13-01-2000	IT PD980169 A1	07-01-2000
		AU 4639799 A	24-01-2000
		CA 2339066 A1	13-01-2000
		EP 1095064 A1	02-05-2001
		WO 0001733 A1	13-01-2000
		JP 2002519481 T	02-07-2002
WO 0054762 A	21-09-2000	AU 3886600 A	04-10-2000
		CA 2365767 A1	21-09-2000
		EP 1162984 A2	19-12-2001
		JP 2002539157 T	19-11-2002
		WO 0054762 A2	21-09-2000
		US 2002169144 A1	14-11-2002
		US 6472379 B1	29-10-2002
WO 0057896 A	05-10-2000	AU 4025500 A	16-10-2000
		CA 2366604 A1	05-10-2000
		EP 1165112 A1	02-01-2002
		JP 2002540161 T	26-11-2002
		WO 0057896 A1	05-10-2000
		US 2003054991 A1	20-03-2003
US 4851521 A	25-07-1989	IT 1203815 B	23-02-1989
		IT 1214658 B	18-01-1990
		AT 135713 T	15-04-1996
		AT 227741 T	15-11-2002
		AU 591501 B2	07-12-1989
		AU 5983686 A	26-02-1987
		CA 1341276 C	31-07-2001
		DE 3650501 D1	25-04-1996
		DE 3650501 T2	21-11-1996
		DE 3650776 D1	19-12-2002
		DK 323686 A	09-01-1987
		EP 0216453 A2	01-04-1987
		EP 0696598 A1	14-02-1996
		ES 2001512 A6	01-06-1988
		FI 862878 A ,B,	09-01-1987
		FI 892710 A ,B,	02-06-1989
		FI 892711 A	02-06-1989

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4851521 A		FI 94767 B	14-07-1995
		HU 42512 A2	28-07-1987
		IE 81120 B1	22-03-2000
		IL 79362 A	31-07-1995
		IN 165582 A1	25-11-1989
		JP 2569012 B2	08-01-1997
		JP 62064802 A	23-03-1987
		KR 8701901 B1	21-10-1987
		NO 862734 A ,B,	09-01-1987
		NO 910295 A	09-01-1987
		NZ 216786 A	26-05-1992
		NZ 233045 A	26-05-1992
		PH 25189 A	27-03-1991
		PT 82941 A ,B	01-08-1986
		SG 90006 A1	23-07-2002
		US 4965353 A	23-10-1990
		US 5202431 A	13-04-1993
		US 5336767 A	09-08-1994
		PH 25383 A	03-06-1991
		PH 25382 A	03-06-1991
		PH 25642 A	21-08-1991
		PH 25643 A	21-08-1991
		ZA 8605071 A	30-09-1987
EP 0466300 A	15-01-1992	US 5143724 A	01-09-1992
		AT 165979 T	15-05-1998
		AU 629467 B2	01-10-1992
		AU 7405591 A	09-01-1992
		CA 2041074 A1	10-01-1992
		DE 69129391 D1	18-06-1998
		DE 69129391 T2	08-10-1998
		DK 466300 T3	07-10-1998
		EP 0466300 A2	15-01-1992
		ES 2117634 T3	16-08-1998
		HK 1011502 A1	09-07-1999
		JP 4261664 A	17-09-1992
		JP 7093943 B	11-10-1995
		US 5399351 A	21-03-1995
		US 5246698 A	21-09-1993